PREVALENCE OF ANTIBIOTIC RESISTANT BACTERIAL ISOLATES AND CHARACTERIZATION OF PLASMID DNA CONFERRING MDR VIA CONJUGATION FROM POTABLE WATER SAMPLES

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ABSTRACT
RO and RO+UV treated water quality of various water coolers and purifiers of Indore region were analyzed for the detection of total coliforms and multiple drug resistant (MDR) bacteria. Most of the samples from only RO water purifier have shown >240 coliforms/100ml while some have 21, 12, 2.2 coliforms respectively. Lactose fermenting and non-lactose fermenting organisms were identified on the basis of biochemical reactions. Mostly E. coli, Klebsiella pneumoniae, Enterobacter aerogenes, Pseudomonas aeruginosa and Proteus sp. organisms were observed to contaminate water. Mueller Hinton Agar Medium was used to check the susceptibility of isolates towards various antibiotics. All the isolates were found to be resistant to Ampicillin. Some isolates were resistant to Streptomycin, Gentamycin, Amikacin, Chloramphenicol, Ampicillin, Ofloxacin and Ciprofloxacin. The percentages of antibiotic resistance of isolates are Escherichia coli (57%), Klebsiella pneumoniae (33%), Enterobacter aerogenes (38%), Pseudomonas aeruginosa (19%) and Proteus sp. (14%). A large number of E. coli isolates (57%) exhibited resistance to Ampicillin (100%), Streptomycin (25%), Gentamycin (25%), Amikacin (16.66%), Chloramphenicol (8.33%), and Ofloxacin (8.33%) antibiotics. Among all the isolates, E. coli (14.25%) was found to be multiple drug resistant bacteria. The extracted plasmid DNA of 1.8kb was isolated from this amp resistant E. coli. The plasmid DNA of amp resistant E. coli bacteria was successfully transferred to amp sensitive bacteria via conjugation process. All these drug resistant bacteria were isolated from RO water purifier which concludes that RO water samples was of poor microbial quality while RO+UV treated water was found to be safest for drinking purpose.

KEYWORDS: Water Purifier, Coliforms, Antibiogram, MDR, Plasmid DNA, Conjugation.

INTRODUCTION
The bacteriological quality of drinking water is of paramount importance and monitoring must be given highest priority (Bharti et al., 2011). The most dangerous form of water pollution occurs when fecal contaminant like Escherichia coli enter the water supply. Contaminants ingested cause many diseases. Examples of such pathogens are Salmonella sp., Shigella sp., Vibrio cholerae and E. coli (Tortora et al., 2002). The quality of potable water and treatment of water borne diseases are critical public health issues. Bacterial contamination of drinking water sources is the most common health risk. Majority of the water sources were not safe for drinking (Solomon et al., 2011). Thereby most of the people rely on RO and RO+UV systems for the purification of water. In RO systems, reverse osmosis treatment is an effective method of reducing the concentration of total dissolved solids (TDS) and many impurities found in water whereas RO membrane can remove all microorganisms while UV kills all remaining bacteria and viruses (Kneen et al., 2005). The Microbiological quality of drinking water has attracted great attention worldwide because of implied public health impacts (Amira et al., 2011). Total and fecal coliforms have been used extensively from many years as indicator for determining the sanitary quality of water sources (Solomon et al., 2011).

Fecal coliforms are a group of bacteria, which are natural inhabitants of the gut of humans and other warm-blooded animals. Escherichia coli (E. coli) is a member of fecal coliforms that contaminate the drinking water from human and animal fecal waste (Ahmed et al., 1996). E. coli is an opportunistic pathogen in neonatal and immunocompromised patients (Annette et al., 1998). Bacteremia, wound infections, urinary tract infection, and gastrointestinal infections are the diseases associated with E. coli and are often fatal in newborns (Raina et al., 1999). Food and water borne out breaks of E. coli have been documented from a number of countries (Ogden et al., 2001; Bartlet et al., 1996). Maintenance of the microbiological quality of water has been used as an important means of preventing waterborne disease (WHO Report, 2008). Antibiotic resistance is becoming a very large problem throughout the world (Bergeron et al., 2015). The occurrence and spread of antibiotic resistant bacteria (ARB) are pressing public health issues worldwide, and aquatic ecosystems are a recognized reservoir for ARB and antibiotic resistance genes (ARGs) (Baquero et al., 2008; Cook et al., 1975; Gonzal et al., 1979; Klare et al., 1995; Kummerer et al., 2004; Martinez et al., 2008; Zhang et al., 2009). Antibiotic resistance in bacteria has been arise from the antibiotic residues which is discharged into the environment when it is not fully metabolized by the body and excreted in its original form.
Characterization of plasmid DNA conferring MDR via conjugation from potable water samples

which results in antibiotic resistant bacteria. Bacteria can easily acquire resistance against those antibiotics and release their antibiotic resistance genes (ARGs) into the environment (Auerbach et al., 2007; Zhang et al., 2009). These released ARGs through genetic transformation can get easily be transferred to the environmental bacteria and pathogens, increasing risks and dangers to environment and human (Liu et al., 2012). This study was aimed to check the prevalence of antibiotic resistant bacterial isolates and characterization of plasmid DNA conferring MDR via conjugation from potable water (RO and RO+UV) samples.

MATERIALS & METHODS

Sample collection
From different locations of Indore city, 30 water samples were collected from various water coolers and purifiers (RO and RO+UV) in non-reactive borosilicate glass/plastic bottles sterilized by autoclaving at 121°C, 30 minutes.

Microbiological analysis of water
Samples were assessed within 24 hrs for the presence of coliforms (Total coliforms and fecal coliforms) by using standard protocol of American Public Health Association (APHA 2005). All samples were analyzed for total coliform count by Most Probable Number (MPN) method. Positive tubes were streaked on MacConkey agar plate to differentiate between lactose fermenting and non-lactose fermenting organisms which were identified on the basis of biochemical reactions.

Antibiotic Resistance Profile
Antibiotic susceptibility test was performed using standardized Kirby Bauer Disc Diffusion Test (Bauer et al., 1966). Mueller Hinton Agar Medium was used to check the susceptibility of isolates towards various antibiotics. The isolated bacterial colonies which were identified by standard biochemical characters were tested against seven commonly used antibiotics, i.e. Streptomycin (10 g), Gentamycin (10 g), Amikacin (30 g), Chloramphenicol (30 g), Ampicillin (10 g), Ofloxacin (5 g) and Ciprofloxacin (5 g). Bacteriological culture media and antibiotic discs of Hi-Media Pvt. Ltd. Mumbai were used.

Isolation of plasmid DNA
Alkaline lysis method was used for purification of plasmid DNA from isolated MDR E. coli bacteria. Agarose gel electrophoresis was performed for the separation of plasmid DNA on the basis of its size and shape.

Conjugation
Lactose fermenting and ampicillin resistant donor E. coli bacterial culture was used for the transfer of plasmid DNA into non lactose fermenting ampicillin sensitive Salmonella typhi bacteria.

RESULTS & DISCUSSION
It was analyzed from 30 investigated samples depicted in Table 1 shows >240 coliforms/100 ml contamination in 40% of RO treated water samples which was highly contaminated and not fit for drinking purpose. 7% and 3% of RO treated water samples, 21 coliforms /100 ml and 12 coliforms /100 ml were observed respectively which shows that the quality of water sample is unsatisfactory. 2.2 coliforms /100 ml were observed in 10% of water samples, which indicates that water sample is unsatisfactory. Zero coliforms were observed in 40% of RO+UV treated water sample. Kumar et al., 2013 also observed that out of 26 water samples of water cooler, 18 samples were found to be unsatisfactory. He reported 90 coliforms /100 ml from water cooler which was non potable. Coliforms identified as Escherichia coli (Figure - 1 & 2), Klebsiella pneumoniae, Enterobacter aerogenes were present in 40%, 23%, 27% of samples, respectively while non lactose fermenting organisms identified as Pseudomonas aeruginosa and Proteus sp. were found in 13%, 10% of samples, respectively. Similar coliforms Escherichia coli, Klebsiella pneumoniae, Enterobacter aerogenes were identified by Pathak et al., 2008, Sapkota et al., 2012 & Bergeron et al., 2015. Escherichia coli, Pseudomonas aeruginosa were detected from potable water in public water supply within Lagos University by Ojo et al., 2008.
Another important work of the present study was to check the prevalence of multiple drug resistance (MDR) in the bacteria isolated from water samples and transferability of the antibiotic resistance markers was also investigated in *Escherichia coli* (MDR) (Figure–3) isolate via conjugation process. The results of the antibiotic susceptibility of the bacteria showed that all the isolates were found to be resistant to Ampicillin while some isolates were resistant to Streptomycin, Gentamycin, Amikacin, Chloramphenicol, Ampicillin, Ofloxacin and Ciprofloxacin (Table 1). Figure 4 shows the percentage of antibiotic resistance of *Escherichia coli* (57%), *Klebsiella pneumoniae* (33%), *Enterobacter aerogenes* (38%), *Pseudomonas aeruginosa* (19%), and *Proteus* sp. (14%). Similar Antibiotic resistant bacteria were observed in treated drinking water by Bergeron et al., 2015. A large number of *E. coli* isolates (57%) exhibited resistance to Ampicillin (100%), Streptomycin (25%), Gentamycin (25%), Amikacin (16.66%), and Chloramphenicol (8.33%), Ofloxacin (8.33%) antibiotics (Figure–5). Among all the isolates, *E. coli* (14.25%) was found to be multiple drug resistant bacteria. Walia et al., 2012 also isolated *E. coli* which was found to be resistant to Amikacin (8%), Streptomycin (49%) and Chloramphenicol (9%). Antibiotic resistant coliform bacteria are common in the intestine of man and as a result of sewage pollution they may become widely disseminated into the environment and transfer antibiotic resistance to other sensitive coliforms or enteric pathogens (Adesoji et al., 2013, Osterblad et al., 2000). Presence and survival of cultivable bacteria in drinking water can act as a vehicle to disseminate virulence genes to other bacteria. This can result in high morbidity and mortality, and the failure of the treatment of life threatening bacterial infections in humans and animals (Walia et al., 2012).

![Confirmatory test for E. coli on MacConkey agar medium plate (sample no. 28)](image1)

**FIGURE 2:** Confirmatory test for *E. coli* on MacConkey agar medium plate (sample no. 28)

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<th><strong>TABLE 1:</strong> Antibiotic resistance pattern of bacterial isolates obtained from water samples</th>
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![Antimicrobial susceptibility test for E. col on Mueller Hinton agar medium plate (sample no. 28)](image2)

**FIGURE 3:** Antimicrobial susceptibility test for *E. coli* on Mueller Hinton agar medium plate (sample no. 28)
In our study the extracted plasmid DNA of 1.8kb was isolated from this amp resistant E. coli. The plasmid DNA of amp resistant E. coli bacteria was successfully transferred to amp sensitive bacteria via conjugation process. Similar work was performed by Walia et al., 2012, who observed that a majority of the multiple antibiotic resistant E. coli isolates contained one or more plasmids (size ranged 1.4 Kb to 40Kb). The ABR (Antibiotic resistance) traits were transferable to other bacteria via conjugation. These data raise an important question about the impact of E. coli containing self-transmissible R-plasmids as a potential reservoir of virulence genes in drinking water.
CONCLUSION
The results of bacteriological analysis of potable water showed that most of the samples were found contaminated and not fit for drinking purpose. Maintenance of RO system is essential for reliable performance. High level of TDS & microorganisms in the system are commonly the cause of fouled membranes. The treated water should be monitored for TDS and the level of any specific contaminants that may affect the quality of water and family’s health. Occurrence and prevalence of these resistant strains in environment is a cause of concern as they can act as a vehicle to disseminate resistance to other bacteria. Much needs to be done to increase awareness of the hazards of drinking contaminated water and of ways to prevent contamination. All these drug resistant bacteria were isolated from RO water purifier which concludes that RO water samples was of poor microbial quality since RO only reduces hardness of water but not concerned with the killing of microorganisms while RO+UV treated water was found to be safest for drinking purpose because it purifies water by ultrafiltration and kills bacteria by UV processes.

REFERENCES


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